

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/52, 14/475, C12N 15/12, 15/63, 15/70, 15/79, A61K 38/17	A1	(11) International Publication Number: WO 99/41284 (43) International Publication Date: 19 August 1999 (19.08.99)
(21) International Application Number: PCT/US99/02898 (22) International Filing Date: 11 February 1999 (11.02.99) (30) Priority Data: 60/075,118 11 February 1998 (11.02.98) US Not furnished 10 February 1999 (10.02.99) US (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). (72) Inventors: WONG, Gordon, G.; 239 Clark Road, Brookline, MA 02146 (US). CLARK, Hilary, F.; 146 Webster Avenue #2, Cambridge, MA 02141 (US). FECHTEL, Kim; 46 Marion Road, Arlington, MA 02174 (US). AGOSTINO, Michael, J.; 26 Wolcott Avenue, Andover, MA 01810 (US). (74) Agent: SPRUNGER, Suzanne, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM (57) Abstract Novel polynucleotides and the proteins encoded thereby are disclosed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

5 SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM

This application is a continuation-in-part of provisional application Ser. No. 60/075,118, filed February 11, 1998, which is incorporated by reference herein.

10

FIELD OF THE INVENTION

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

15

BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the
20 past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which
25 isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the
30 cell or tissue source in the case of PCR-based techniques. It is to these proteins and the polynucleotides encoding them that the present invention is directed.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 29 to nucleotide 253;
- (c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone ya15_1 deposited under accession number
10 ATCC 98650;
- (d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;
- (e) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone ya15_1 deposited under accession number ATCC
15 98650;
- (f) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
- 20 (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:2;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;
- 25 (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ;
- (k) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(h); and
- (l) a polynucleotide that hybridizes under stringent conditions to any
30 one of the polynucleotides specified in (a)-(h) and that has a length that is at least 25% of the length of SEQ ID NO:1.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 29 to nucleotide 253; the nucleotide sequence of the full-length protein coding sequence of clone ya15_1 deposited under accession number ATCC 98650;

or the nucleotide sequence of a mature protein coding sequence of clone ya15_1 deposited under accession number ATCC 98650. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650. In further preferred
5 embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:2, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having
10 biological activity, the fragment comprising the amino acid sequence from amino acid 32 to amino acid 41 of SEQ ID NO:2.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:1.

Further embodiments of the invention provide isolated polynucleotides produced
15 according to a process selected from the group consisting of:

- (a) a process comprising the steps of:
 - (i) preparing one or more polynucleotide probes that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
 - 20 (aa) SEQ ID NO:1, but excluding the poly(A) tail at the 3' end of SEQ ID NO:1; and
 - (ab) the nucleotide sequence of the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;
 - (ii) hybridizing said probe(s) to human genomic DNA in
25 conditions at least as stringent as 4X SSC at 50 degrees C; and
 - (iii) isolating the DNA polynucleotides detected with the probe(s);
- and
- (b) a process comprising the steps of:
 - 30 (i) preparing one or more polynucleotide primers that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
 - (ba) SEQ ID NO:1, but excluding the poly(A) tail at the 3' end of SEQ ID NO:1; and

- (bb) the nucleotide sequence of the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;
- (ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;
- 5 (iii) amplifying human DNA sequences; and
- (iv) isolating the polynucleotide products of step (b)(iii).

Preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:1, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:1 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:1, but excluding the poly(A) tail at the 3' end of SEQ ID NO:1. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:1 from nucleotide 29 to nucleotide 253, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:1 from nucleotide 29 to nucleotide 253, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:1 from nucleotide 29 to nucleotide 253.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) a fragment of the amino acid sequence of SEQ ID NO:2, the fragment comprising eight contiguous amino acids of SEQ ID NO:2; and
- (c) the amino acid sequence encoded by the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:2. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:2, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment comprising the amino acid sequence from amino acid 32 to amino acid 41 of SEQ ID NO:2.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;
- 5 (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 151 to nucleotide 288;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 196 to nucleotide 288;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone ya24_1 deposited under accession number ATCC 98650;
- 10 (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone ya24_1 deposited under accession number ATCC 98650;
- 15 (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;
- 20 (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:4;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- 25 (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ;
- (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i); and
- 30 (m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i) and that has a length that is at least 25% of the length of SEQ ID NO:3.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:3 from nucleotide 151 to nucleotide 288; the nucleotide sequence of SEQ ID NO:3 from

nucleotide 196 to nucleotide 288; the nucleotide sequence of the full-length protein coding sequence of clone ya24_1 deposited under accession number ATCC 98650; or the nucleotide sequence of a mature protein coding sequence of clone ya24_1 deposited under accession number ATCC 98650. In other preferred embodiments, the polynucleotide
5 encodes the full-length or a mature protein encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous
10 amino acids of SEQ ID NO:4, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment comprising the amino acid sequence from amino acid 18 to amino acid 27 of SEQ ID NO:4.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ
15 ID NO:3.

Further embodiments of the invention provide isolated polynucleotides produced according to a process selected from the group consisting of:

- (a) a process comprising the steps of:
 - (i) preparing one or more polynucleotide probes that hybridize
20 in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
 - (aa) SEQ ID NO:3, but excluding the poly(A) tail at the 3' end of SEQ ID NO:3; and
 - (ab) the nucleotide sequence of the cDNA insert of clone
25 ya24_1 deposited under accession number ATCC 98650;
 - (ii) hybridizing said probe(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C; and
 - (iii) isolating the DNA polynucleotides detected with the probe(s);
- 30 and
- (b) a process comprising the steps of:
 - (i) preparing one or more polynucleotide primers that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(ba) SEQ ID NO:3, but excluding the poly(A) tail at the 3' end of SEQ ID NO:3; and

(bb) the nucleotide sequence of the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;

5 (ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;

(iii) amplifying human DNA sequences; and

(iv) isolating the polynucleotide products of step (b)(iii).

Preferably the polynucleotide isolated according to the above process comprises a
10 nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:3, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:3 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:3, but excluding the poly(A) tail at the 3' end of SEQ ID NO:3. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the
15 cDNA sequence of SEQ ID NO:3 from nucleotide 151 to nucleotide 288, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:3 from nucleotide 151 to nucleotide 288, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:3 from nucleotide 151 to nucleotide 288. Also preferably the polynucleotide isolated according to the above
20 process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:3 from nucleotide 196 to nucleotide 288, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:3 from nucleotide 196 to nucleotide 288, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:3 from nucleotide 196 to nucleotide 288.

25 In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) a fragment of the amino acid sequence of SEQ ID NO:4, the
30 fragment comprising eight contiguous amino acids of SEQ ID NO:4; and

(c) the amino acid sequence encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:4. In further preferred

embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:4, or a protein comprising a fragment of the amino acid sequence of SEQ
5 ID NO:4 having biological activity, the fragment comprising the amino acid sequence from amino acid 18 to amino acid 27 of SEQ ID NO:4.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 10 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 615 to nucleotide 908;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 774 to nucleotide 908;
- 15 (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yb42_1 deposited under accession number ATCC 98650;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
- 20 (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone yb42_1 deposited under accession number ATCC 98650;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
- 25 (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:6;
- 30 (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ;

(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i); and

(m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i) and that has a length that is at least 25% of the length of SEQ ID NO:5.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:5 from nucleotide 615 to nucleotide 908; the nucleotide sequence of SEQ ID NO:5 from nucleotide 774 to nucleotide 908; the nucleotide sequence of the full-length protein coding sequence of clone yb42_1 deposited under accession number ATCC 98650; or the nucleotide sequence of a mature protein coding sequence of clone yb42_1 deposited under accession number ATCC 98650. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:6, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment comprising the amino acid sequence from amino acid 44 to amino acid 53 of SEQ ID NO:6.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:5.

Further embodiments of the invention provide isolated polynucleotides produced according to a process selected from the group consisting of:

- (a) a process comprising the steps of:
- (i) preparing one or more polynucleotide probes that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
 - (aa) SEQ ID NO:5, but excluding the poly(A) tail at the 3' end of SEQ ID NO:5; and
 - (ab) the nucleotide sequence of the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
 - (ii) hybridizing said probe(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C; and

(iii) isolating the DNA polynucleotides detected with the probe(s);

and

(b) a process comprising the steps of:

5 (i) preparing one or more polynucleotide primers that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(ba) SEQ ID NO:5, but excluding the poly(A) tail at the 3' end of SEQ ID NO:5; and

10 (bb) the nucleotide sequence of the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;

(ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;

(iii) amplifying human DNA sequences; and

15 (iv) isolating the polynucleotide products of step (b)(iii).

Preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:5, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:5 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:5, but excluding the poly(A) tail at the 3' end of SEQ ID NO:5. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:5 from nucleotide 615 to nucleotide 908, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:5 from nucleotide 615 to nucleotide 908, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:5 from nucleotide 615 to nucleotide 908. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:5 from nucleotide 774 to nucleotide 908, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:5 from nucleotide 774 to nucleotide 908, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:5 from nucleotide 774 to nucleotide 908.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
 - (b) a fragment of the amino acid sequence of SEQ ID NO:6, the fragment comprising eight contiguous amino acids of SEQ ID NO:6; and
 - (c) the amino acid sequence encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
- 5 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:6. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment preferably
- 10 comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:6, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment comprising the amino acid sequence from amino acid 44 to amino acid 53 of SEQ ID NO:6.

In one embodiment, the present invention provides a composition comprising an

15 isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 1203 to nucleotide 2327;
- 20 (c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yc9_1 deposited under accession number ATCC 98650;
- (d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650;
- 25 (e) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone yc9_1 deposited under accession number ATCC 98650;
- (f) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650;
- 30 (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;
- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:8;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ;

5 (k) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(h); and

(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(h) and that has a length that is at least 25% of the length of SEQ ID NO:7.

10 Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:7 from nucleotide 1203 to nucleotide 2327; the nucleotide sequence of the full-length protein coding sequence of clone yc9_1 deposited under accession number ATCC 98650; or the nucleotide sequence of a mature protein coding sequence of clone yc9_1 deposited under accession number ATCC 98650. In other preferred embodiments, the
15 polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment preferably comprising eight (more preferably twenty, most
20 preferably thirty) contiguous amino acids of SEQ ID NO:8, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising the amino acid sequence from amino acid 182 to amino acid 191 of SEQ ID NO:8.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ
25 ID NO:7.

Further embodiments of the invention provide isolated polynucleotides produced according to a process selected from the group consisting of:

(a) a process comprising the steps of:

30 (i) preparing one or more polynucleotide probes that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(aa) SEQ ID NO:7, but excluding the poly(A) tail at the 3' end of SEQ ID NO:7; and

- (ab) the nucleotide sequence of the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650;
- (ii) hybridizing said probe(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C; and
- 5 (iii) isolating the DNA polynucleotides detected with the probe(s);
- and
- (b) a process comprising the steps of:
- (i) preparing one or more polynucleotide primers that
- 10 hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
- (ba) SEQ ID NO:7, but excluding the poly(A) tail at the 3' end of SEQ ID NO:7; and
- (bb) the nucleotide sequence of the cDNA insert of clone
- 15 yc9_1 deposited under accession number ATCC 98650;
- (ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;
- (iii) amplifying human DNA sequences; and
- (iv) isolating the polynucleotide products of step (b)(iii).
- 20 Preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:7, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:7 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:7, but excluding the poly(A) tail at the 3' end of SEQ ID NO:7. Also preferably the polynucleotide isolated
- 25 according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:7 from nucleotide 1203 to nucleotide 2327, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:7 from nucleotide 1203 to nucleotide 2327, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:7 from nucleotide 1203 to
- 30 nucleotide 2327.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:8;

- (b) a fragment of the amino acid sequence of SEQ ID NO:8, the fragment comprising eight contiguous amino acids of SEQ ID NO:8; and
- (c) the amino acid sequence encoded by the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650;
- 5 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:8. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids
- 10 of SEQ ID NO:8, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising the amino acid sequence from amino acid 182 to amino acid 191 of SEQ ID NO:8.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 15 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 230 to nucleotide 823;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID
- 20 NO:9 from nucleotide 584 to nucleotide 823;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yc19_1 deposited under accession number ATCC 98650;
- (e) a polynucleotide encoding the full-length protein encoded by the
- 25 cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone yc19_1 deposited under accession number ATCC 98650;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA
- 30 insert of clone yc19_1 deposited under accession number ATCC 98650;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:10;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ;

(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i); and

(m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i) and that has a length that is at least 25% of the length of SEQ ID NO:9.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:9 from nucleotide 230 to nucleotide 823; the nucleotide sequence of SEQ ID NO:9 from nucleotide 584 to nucleotide 823; the nucleotide sequence of the full-length protein coding sequence of clone yc19_1 deposited under accession number ATCC 98650; or the nucleotide sequence of a mature protein coding sequence of clone yc19_1 deposited under accession number ATCC 98650. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:10, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment comprising the amino acid sequence from amino acid 94 to amino acid 103 of SEQ ID NO:10.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:9.

Further embodiments of the invention provide isolated polynucleotides produced according to a process selected from the group consisting of:

(a) a process comprising the steps of:

(i) preparing one or more polynucleotide probes that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(aa) SEQ ID NO:9, but excluding the poly(A) tail at the 3' end of SEQ ID NO:9; and

(ab) the nucleotide sequence of the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;

(ii) hybridizing said probe(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C; and

(iii) isolating the DNA polynucleotides detected with the probe(s);

and

(b) a process comprising the steps of:

(i) preparing one or more polynucleotide primers that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(ba) SEQ ID NO:9, but excluding the poly(A) tail at the 3' end of SEQ ID NO:9; and

(bb) the nucleotide sequence of the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;

(ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;

(iii) amplifying human DNA sequences; and

(iv) isolating the polynucleotide products of step (b)(iii).

Preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:9, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:9 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:9, but excluding the poly(A) tail at the 3' end of SEQ ID NO:9. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:9 from nucleotide 230 to nucleotide 823, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:9 from nucleotide 230 to nucleotide 823, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:9 from nucleotide 230 to

nucleotide 823. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:9 from nucleotide 584 to nucleotide 823, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:9 from nucleotide 584 to nucleotide 823, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:9 from nucleotide 584 to nucleotide 823.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- 10 (a) the amino acid sequence of SEQ ID NO:10;
 - (b) a fragment of the amino acid sequence of SEQ ID NO:10, the fragment comprising eight contiguous amino acids of SEQ ID NO:10; and
 - (c) the amino acid sequence encoded by the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;
- 15 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:10. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids
- 20 of SEQ ID NO:10, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment comprising the amino acid sequence from amino acid 94 to amino acid 103 of SEQ ID NO:10.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 25 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 292 to nucleotide 534;
- (c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yc20_1 deposited under accession number ATCC 98650;
- 30 (d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650;

- (e) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone yc20_1 deposited under accession number ATCC 98650;
- (f) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
- (h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:12;
- (i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;
- (j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ;
- (k) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(h); and
- (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(h) and that has a length that is at least 25% of the length of SEQ ID NO:11.
- Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:11 from nucleotide 292 to nucleotide 534; the nucleotide sequence of the full-length protein coding sequence of clone yc20_1 deposited under accession number ATCC 98650; or the nucleotide sequence of a mature protein coding sequence of clone yc20_1 deposited under accession number ATCC 98650. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:12, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment comprising the amino acid sequence from amino acid 35 to amino acid 44 of SEQ ID NO:12.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:11.

Further embodiments of the invention provide isolated polynucleotides produced according to a process selected from the group consisting of:

- 5 (a) a process comprising the steps of:
- (i) preparing one or more polynucleotide probes that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
 - 10 (aa) SEQ ID NO:11, but excluding the poly(A) tail at the 3' end of SEQ ID NO:11; and
 - (ab) the nucleotide sequence of the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650;
 - (ii) hybridizing said probe(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C; and
 - 15 (iii) isolating the DNA polynucleotides detected with the probe(s);
- and
- (b) a process comprising the steps of:
- (i) preparing one or more polynucleotide primers that
 - 20 hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:
 - (ba) SEQ ID NO:11, but excluding the poly(A) tail at the 3' end of SEQ ID NO:11; and
 - (bb) the nucleotide sequence of the cDNA insert of clone
 - 25 yc20_1 deposited under accession number ATCC 98650;
 - (ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;
 - (iii) amplifying human DNA sequences; and
 - (iv) isolating the polynucleotide products of step (b)(iii).
- 30 Preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:11, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:11 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:11, but excluding the poly(A) tail at the 3' end of SEQ ID NO:11. Also preferably the

polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:11 from nucleotide 292 to nucleotide 534, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:11 from nucleotide 292 to nucleotide 534, to a nucleotide
5 sequence corresponding to the 3' end of said sequence of SEQ ID NO:11 from nucleotide 292 to nucleotide 534.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- 10 (a) the amino acid sequence of SEQ ID NO:12;
 - (b) a fragment of the amino acid sequence of SEQ ID NO:12, the fragment comprising eight contiguous amino acids of SEQ ID NO:12; and
 - (c) the amino acid sequence encoded by the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650;
- 15 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:12. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids
20 of SEQ ID NO:12, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment comprising the amino acid sequence from amino acid 35 to amino acid 44 of SEQ ID NO:12.

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial,
25 yeast, insect and mammalian cells, transformed with such polynucleotide compositions. Also provided by the present invention are organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein.

Processes are also provided for producing a protein, which comprise:

- 30 (a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and
- (b) purifying the protein from the culture.

The protein produced according to such methods is also provided by the present invention.

Protein compositions of the present invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

5 Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Figures 1A and 1B are schematic representations of the pED6 and pNOTs vectors, respectively, used for deposit of clones disclosed herein.

DETAILED DESCRIPTION

ISOLATED PROTEINS AND POLYNUCLEOTIDES

15 Nucleotide and amino acid sequences, as presently determined, are reported below for each clone and protein disclosed in the present application. The nucleotide sequence of each clone can readily be determined by sequencing of the deposited clone in accordance with known methods. The predicted amino acid sequence (both full-length and mature forms) can then be determined from such nucleotide sequence. The amino
20 acid sequence of the protein encoded by a particular clone can also be determined by expression of the clone in a suitable host cell, collecting the protein and determining its sequence. For each disclosed protein applicants have identified what they have determined to be the reading frame best identifiable with sequence information available at the time of filing.

25 As used herein a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation
30 proteins which are transported across the membrane of the endoplasmic reticulum.

Clone "ya15_1"

A polynucleotide of the present invention has been identified as clone "ya15_1". ya15_1 was isolated from a human adult testes cDNA library and was identified as

encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. ya15_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "ya15_1 protein").

The nucleotide sequence of ya15_1 as presently determined is reported in SEQ ID NO:1, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the ya15_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:2. Amino acids 8 to 20 of SEQ ID NO:2 are a possible leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 21. Due to the hydrophobic nature of the predicted leader/signal sequence, it is likely to act as a transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the ya15_1 protein.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone ya15_1 should be approximately 1100 bp.

The nucleotide sequence disclosed herein for ya15_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. ya15_1 demonstrated at least some similarity with sequences identified as Z94056 (Human DNA sequence from PAC 436M11 on chromosome Xp22.11-22.2; contains a serine threonine protein phosphatase gene, ESTs and STSs). Based upon sequence similarity, ya15_1 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the ya15_1 protein sequence centered around amino acid 40 of SEQ ID NO:2. The nucleotide sequence of ya15_1 indicates that it may contain an Alu repetitive element.

Clone "ya24_1"

A polynucleotide of the present invention has been identified as clone "ya24_1". ya24_1 was isolated from a human adult testes cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. ya24_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "ya24_1 protein").

The nucleotide sequence of ya24_1 as presently determined is reported in SEQ ID NO:3, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the ya24_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:4. Amino acids 3 to 15 of

SEQ ID NO:4 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 16. Due to the hydrophobic nature of the predicted leader/signal sequence, it is likely to act as a transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the ya24_1 protein.

5 The EcoRI/NotI restriction fragment obtainable from the deposit containing clone ya24_1 should be approximately 750 bp.

 The nucleotide sequence disclosed herein for ya24_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. ya24_1 demonstrated at least some similarity with sequences
10 identified as AA537299 (vk46d03.r1 Soares mouse mammary gland NbMMG Mus musculus cDNA clone 949637 5'). Based upon sequence similarity, ya24_1 proteins and each similar protein or peptide may share at least some activity.

Clone "yb42_1"

15 A polynucleotide of the present invention has been identified as clone "yb42_1". yb42_1 was isolated from a human fetal brain cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. yb42_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "yb42_1 protein").

20 The nucleotide sequence of yb42_1 as presently determined is reported in SEQ ID NO:5, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the yb42_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:6. Amino acids 41 to 53 of SEQ ID NO:6 are a predicted leader/signal sequence, with the predicted
25 mature amino acid sequence beginning at amino acid 54. Due to the hydrophobic nature of the predicted leader/signal sequence, it is likely to act as a transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the yb42_1 protein.

 Another potential yb42_1 reading frame and predicted amino acid sequence is
30 encoded by basepairs 1879 to 2220 of SEQ ID NO:5 and is reported in SEQ ID NO:19; amino acids 54 to 66 of SEQ ID NO:19 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 67 of SEQ ID NO:19. Due to the hydrophobic nature of this predicted leader/signal sequence, it is likely to act as a

transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the protein of SEQ ID NO:19.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone yb42_1 should be approximately 3900 bp.

5 The nucleotide sequence disclosed herein for yb42_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. yb42_1 demonstrated at least some similarity with sequences identified as AA213992 (zn58d08.s1 Stratagene muscle 937209 Homo sapiens cDNA clone 562383 3'). Based upon sequence similarity, yb42_1 proteins and each similar protein or
10 peptide may share at least some activity. The nucleotide sequence of yb42_1 indicates that it may contain at least one MIR repeat sequence.

Clone "yc9_1"

A polynucleotide of the present invention has been identified as clone "yc9_1".
15 yc9_1 was isolated from a human fetal kidney (293 cell line) cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. yc9_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "yc9_1 protein").

20 The nucleotide sequence of yc9_1 as presently determined is reported in SEQ ID NO:7, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the yc9_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:8.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone
25 yc9_1 should be approximately 3300 bp.

The nucleotide sequence disclosed herein for yc9_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. yc9_1 demonstrated at least some similarity with sequences identified as AA588539 (nm94a07.s1 NCI_CGAP_Co9 Homo sapiens cDNA clone
30 IMAGE:1075860) and N47418 (yy88e12.r1 Homo sapiens cDNA clone 280654 5'). The predicted amino acid sequence disclosed herein for yc9_1 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted yc9_1 protein demonstrated at least some similarity to sequences identified as X97196 (D. melanogaster X gene). Based upon sequence similarity, yc9_1 proteins and

each similar protein or peptide may share at least some activity. The TopPredII computer program predicts two potential transmembrane domains within the yc9_1 protein sequence, centered around amino acids 80 and 200 of SEQ ID NO:8, respectively.

5 Clone "yc19_1"

A polynucleotide of the present invention has been identified as clone "yc19_1". yc19_1 was isolated from a human fetal kidney (293 cell line) cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. yc19_1 is a full-length clone,
10 including the entire coding sequence of a secreted protein (also referred to herein as "yc19_1 protein").

The nucleotide sequence of yc19_1 as presently determined is reported in SEQ ID NO:9, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the yc19_1 protein corresponding
15 to the foregoing nucleotide sequence is reported in SEQ ID NO:10. Amino acids 106 to 118 of SEQ ID NO:10 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 119. Due to the hydrophobic nature of the predicted leader/signal sequence, it is likely to act as a transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the yc19_1
20 protein.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone yc19_1 should be approximately 1400 bp.

The nucleotide sequence disclosed herein for yc19_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and
25 FASTA search protocols. yc19_1 demonstrated at least some similarity with sequences identified as AA126002 (zl85a08.s1 Stratagene colon (#937204) Homo sapiens cDNA clone 511382 3') and AA307000 (EST177917 Colon carcinoma (HCC) cell line Homo sapiens cDNA 5' end). The predicted amino acid sequence disclosed herein for yc19_1 was searched against the GenPept and GeneSeq amino acid sequence databases using the
30 BLASTX search protocol. The predicted yc19_1 protein demonstrated at least some similarity to sequences identified as X83742 (MAP kinase phosphatase [Xenopus laevis]), and to several tyrosine phosphatases from other species. Based upon sequence similarity, yc19_1 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts an additional potential transmembrane domain

within the yc19_1 protein sequence centered around amino acid 43 of SEQ ID NO:10; this region (amino acids 30 to 42 of SEQ ID NO:10) may also be a leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 43.

yc19_1 protein was expressed in a COS cell expression system, and an expressed
5 protein band of approximately 23 kDa was detected in conditioned medium and membrane fractions using SDS polyacrylamide gel electrophoresis.

Clone "yc20_1"

A polynucleotide of the present invention has been identified as clone "yc20_1".
10 yc20_1 was isolated from a human fetal kidney (293 cell line) cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. yc20_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "yc20_1 protein").

15 The nucleotide sequence of yc20_1 as presently determined is reported in SEQ ID NO:11, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the yc20_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:12.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone
20 yc20_1 should be approximately 1450 bp.

The nucleotide sequence disclosed herein for yc20_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. yc20_1 demonstrated at least some similarity with sequences identified as AA447839 (aa18c12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone
25 813622 5'), N33405 (yy41e10.s1 Homo sapiens cDNA clone 273834 3'), and T19519 (Human gene signature HUMGS00580). Based upon sequence similarity, yc20_1 proteins and each similar protein or peptide may share at least some activity.

Deposit of Clones

30 Clones ya15_1, ya24_1, yb42_1, yc9_1, yc19_1, and yc20_1 were deposited on February 11, 1998 with the American Type Culture Collection (10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number ATCC 98650, from which each clone comprising a particular polynucleotide is obtainable. All restrictions on the

availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b), and the term of the deposit will comply with 37 C.F.R. § 1.806.

Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit. Each clone can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' site, EcoRI; 3' site, NotI) to produce the appropriate fragment for such clone. Each clone was deposited in either the pED6 or pNOTs vector depicted in Figures 1A and 1B, respectively. The pED6dpc2 vector ("pED6") was derived from pED6dpc1 by insertion of a new polylinker to facilitate cDNA cloning (Kaufman *et al.*, 1991, *Nucleic Acids Res.* 19: 4485-4490); the pNOTs vector was derived from pMT2 (Kaufman *et al.*, 1989, *Mol. Cell. Biol.* 9: 946-958) by deletion of the DHFR sequences, insertion of a new polylinker, and insertion of the M13 origin of replication in the ClaI site. In some instances, the deposited clone can become "flipped" (i.e., in the reverse orientation) in the deposited isolate. In such instances, the cDNA insert can still be isolated by digestion with EcoRI and NotI. However, NotI will then produce the 5' site and EcoRI will produce the 3' site for placement of the cDNA in proper orientation for expression in a suitable vector. The cDNA may also be expressed from the vectors in which they were deposited.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences. The sequence of an oligonucleotide probe that was used to isolate or to sequence each full-length clone is identified below, and should be most reliable in isolating the clone of interest.

<u>Clone</u>	<u>Probe Sequence</u>
ya15_1	SEQ ID NO:13
ya24_1	SEQ ID NO:14
30 yb42_1	SEQ ID NO:15
yc9_1	SEQ ID NO:16
yc19_1	SEQ ID NO:17
yc20_1	SEQ ID NO:18

In the sequences listed above which include an N at position 2, that position is occupied in preferred probes/primers by a biotinylated phosphoramidite residue rather than a nucleotide (such as, for example, that produced by use of biotin phosphoramidite (1-dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite) (Glen Research, cat. no. 10-1953)).

The design of the oligonucleotide probe should preferably follow these parameters:

- (a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;
- (b) It should be designed to have a T_m of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).

The oligonucleotide should preferably be labeled with γ -³²P ATP (specific activity 6000 Ci/mmol) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4e+6 dpm/pmol.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 µg/ml. The culture should preferably be grown to saturation at 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 µg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 µg/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1e+6 dpm/mL. The filter is then preferably

incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for 30 minutes to 1 hour is optional. The
5 filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis,
10 hybridization analysis, or DNA sequencing.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, *Bio/Technology* 10, 773-778 (1992) and in R.S.
15 McDowell, *et al.*, *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion
20 could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence
25 listing by translation of the nucleotide sequence of each disclosed clone. The mature form(s) of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell or other host cell. The sequence(s) of the mature form(s) of the protein may also be determinable from the amino acid sequence of the full-length form.

30 The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited

to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed
5 sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

The chromosomal location corresponding to the polynucleotide sequences
10 disclosed herein may also be determined, for example by hybridizing appropriately labeled polynucleotides of the present invention to chromosomes *in situ*. It may also be possible to determine the corresponding chromosomal location for a disclosed polynucleotide by identifying significantly similar nucleotide sequences in public databases, such as expressed sequence tags (ESTs), that have already been mapped to
15 particular chromosomal locations. For at least some of the polynucleotide sequences disclosed herein, public database sequences having at least some similarity to the polynucleotide of the present invention have been listed by database accession number. Searches using the GenBank accession numbers of these public database sequences can then be performed at an Internet site provided by the National Center for Biotechnology
20 Information having the address <http://www.ncbi.nlm.nih.gov/UniGene/>, in order to identify "UniGene clusters" of overlapping sequences. Many of the "UniGene clusters" so identified will already have been mapped to particular chromosomal sites.

Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The
25 desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, *Trends Pharmacol. Sci.* 15(7): 250-254; Lavarosky *et al.*, 1997, *Biochem. Mol. Med.* 62(1): 11-22; and Hampel, 1998, *Prog. Nucleic Acid Res. Mol. Biol.* 58: 1-39; all of which are incorporated by reference herein). Transgenic animals that have
30 multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are

also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through
5 deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, *Bioessays* 14(9): 629-633; Zwaal *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90(16): 7431-7435; Clark *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination,
10 preferably detected by positive/negative genetic selection strategies (Mansour *et al.*, 1988, *Nature* 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614,396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for
15 the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms, part
20 or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information. For example, the TopPredII computer program can be used to predict the location of
25 transmembrane domains in an amino acid sequence, domains which are described by the location of the center of the transmembrane domain, with at least ten transmembrane amino acids on each side of the reported central residue(s).

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25% (more preferably at least 50%, and most
30 preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are

proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

5 In particular, sequence identity may be determined using WU-BLAST (Washington University BLAST) version 2.0 software, which builds upon WU-BLAST version 1.4, which in turn is based on the public domain NCBI-BLAST version 1.4 (Altschul and Gish, 1996, Local alignment statistics, Doolittle *ed.*, *Methods in Enzymology* 266: 460-480; Altschul *et al.*, 1990, Basic local alignment search tool, *Journal of*
10 *Molecular Biology* 215: 403-410; Gish and States, 1993, Identification of protein coding regions by database similarity search, *Nature Genetics* 3: 266-272; Karlin and Altschul, 1993, Applications and statistics for multiple high-scoring segments in molecular sequences, *Proc. Natl. Acad. Sci. USA* 90: 5873-5877; all of which are incorporated by reference herein). WU-BLAST version 2.0 executable programs for several UNIX
15 platforms can be downloaded from <ftp://blast.wustl.edu/blast/executables>. The complete suite of search programs (BLASTP, BLASTN, BLASTX, TBLASTN, and TBLASTX) is provided at that site, in addition to several support programs. WU-BLAST 2.0 is copyrighted and may not be sold or redistributed in any form or manner without the express written consent of the author; but the posted executables may otherwise be freely
20 used for commercial, nonprofit, or academic purposes. In all search programs in the suite -- BLASTP, BLASTN, BLASTX, TBLASTN and TBLASTX -- the gapped alignment routines are integral to the database search itself, and thus yield much better sensitivity and selectivity while producing the more easily interpreted output. Gapping can optionally be turned off in all of these programs, if desired. The default penalty (Q) for a gap of length
25 one is Q=9 for proteins and BLASTP, and Q=10 for BLASTN, but may be changed to any integer value including zero, one through eight, nine, ten, eleven, twelve through twenty, twenty-one through fifty, fifty-one through one hundred, etc. The default per-residue penalty for extending a gap (R) is R=2 for proteins and BLASTP, and R=10 for BLASTN, but may be changed to any integer value including zero, one, two, three, four, five, six,
30 seven, eight, nine, ten, eleven, twelve through twenty, twenty-one through fifty, fifty-one through one hundred, etc. Any combination of values for Q and R can be used in order to align sequences so as to maximize overlap and identity while minimizing sequence gaps.

The default amino acid comparison matrix is BLOSUM62, but other amino acid comparison matrices such as PAM can be utilized.

Species homologues of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide. Preferably, polynucleotide species homologues have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% identity) with the given polynucleotide, and protein species homologues have at least 30% sequence identity (more preferably, at least 45% identity; most preferably at least 60% identity) with the given protein, where sequence identity is determined by comparing the nucleotide sequences of the polynucleotides or the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Species homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. Preferably, species homologues are those isolated from mammalian species. Most preferably, species homologues are those isolated from certain mammalian species such as, for example, *Pan troglodytes*, *Gorilla gorilla*, *Pongo pygmaeus*, *Hylobates concolor*, *Macaca mulatta*, *Papio papio*, *Papio hamadryas*, *Cercopithecus aethiops*, *Cebus capucinus*, *Aotus trivirgatus*, *Sanguinus oedipus*, *Microcebus murinus*, *Mus musculus*, *Rattus norvegicus*, *Cricetulus griseus*, *Felis catus*, *Mustela vison*, *Canis familiaris*, *Oryctolagus cuniculus*, *Bos taurus*, *Ovis aries*, *Sus scrofa*, and *Equus caballus*, for which genetic maps have been created allowing the identification of syntenic relationships between the genomic organization of genes in one species and the genomic organization of the related genes in another species (O'Brien and Seuáñez, 1988, *Ann. Rev. Genet.* 22: 323-351; O'Brien *et al.*, 1993, *Nature Genetics* 3:103-112; Johansson *et al.*, 1995, *Genomics* 25: 682-690; Lyons *et al.*, 1997, *Nature Genetics* 15: 47-56; O'Brien *et al.*, 1997, *Trends in Genetics* 13(10): 393-399; Carver and Stubbs, 1997, *Genome Research* 7:1123-1137; all of which are incorporated by reference herein).

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotides which also encode proteins which are identical or have significantly similar sequences to those encoded by the disclosed polynucleotides. Preferably, allelic variants have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90%

identity) with the given polynucleotide, where sequence identity is determined by comparing the nucleotide sequences of the polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps. Allelic variants may be isolated and identified by making suitable probes or primers from the sequences provided herein and
5 screening a suitable nucleic acid source from individuals of the appropriate species.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides that hybridize under reduced stringency conditions, more preferably stringent conditions, and most preferably highly
10 stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

	Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp) [‡]	Hybridization Temperature and Buffer [†]	Wash Temperature and Buffer [†]
5	A	DNA:DNA	≥ 50	65°C; 1xSSC -or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
	B	DNA:DNA	<50	T _B *; 1xSSC	T _B *; 1xSSC
	C	DNA:RNA	≥ 50	67°C; 1xSSC -or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
	D	DNA:RNA	<50	T _D *; 1xSSC	T _D *; 1xSSC
	E	RNA:RNA	≥ 50	70°C; 1xSSC -or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
	F	RNA:RNA	<50	T _F *; 1xSSC	T _F *; 1xSSC
10	G	DNA:DNA	≥ 50	65°C; 4xSSC -or- 42°C; 4xSSC, 50% formamide	65°C; 1xSSC
	H	DNA:DNA	<50	T _H *; 4xSSC	T _H *; 4xSSC
	I	DNA:RNA	≥ 50	67°C; 4xSSC -or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
	J	DNA:RNA	<50	T _J *; 4xSSC	T _J *; 4xSSC
	K	RNA:RNA	≥ 50	70°C; 4xSSC -or- 50°C; 4xSSC, 50% formamide	67°C; 1xSSC
	L	RNA:RNA	<50	T _L *; 2xSSC	T _L *; 2xSSC
15	M	DNA:DNA	≥ 50	50°C; 4xSSC -or- 40°C; 6xSSC, 50% formamide	50°C; 2xSSC
	N	DNA:DNA	<50	T _N *; 6xSSC	T _N *; 6xSSC
	O	DNA:RNA	≥ 50	55°C; 4xSSC -or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
	P	DNA:RNA	<50	T _P *; 6xSSC	T _P *; 6xSSC
	Q	RNA:RNA	≥ 50	60°C; 4xSSC -or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
	R	RNA:RNA	<50	T _R *; 4xSSC	T _R *; 4xSSC

[‡] The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

[†] SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

*T_B - T_R: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6(log₁₀[Na⁺]) + 0.41(%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na⁺] is the concentration of sodium ions in the hybridization buffer ([Na⁺] for 1xSSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and *Current Protocols in Molecular Biology*, 1995, F.M. Ausubel et al., eds.,
5 John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or
10 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

The isolated polynucleotide of the invention may be operably linked to an
15 expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably
20 linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the
25 protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

30 Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial

strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLabs (Beverly, MA), Pharmacia (Piscataway, NJ) and Invitrogen Corporation (Carlsbad, CA), respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from the Eastman Kodak Company (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to
5 provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which
10 are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by
15 virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement,
20 insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, substitution, replacement,
25 insertion or deletion retains the desired activity of the protein.
30

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art

given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

USES AND BIOLOGICAL ACTIVITY

5 The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies
10 or vectors suitable for introduction of DNA).

Research Uses and Utilities

 The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express
15 recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare
20 with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for
25 examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, those
30 described in Gyuris *et al.*, 1993, *Cell* 75: 791-803 and in Rossi *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94: 8405-8410, all of which are incorporated by reference herein) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine
5 levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the
10 protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent
15 grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to
20 Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein
25 or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention
30 can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may

induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is
5 evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

10 Assays for T-cell or thymocyte proliferation include without limitation those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., *J. Immunol.* 137:3494-3500, 1986;
15 Bertagnolli et al., *J. Immunol.* 145:1706-1712, 1990; Bertagnolli et al., *Cellular Immunology* 133:327-341, 1991; Bertagnolli, et al., *J. Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell
20 stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ , Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic
25 cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., *J. Exp. Med.* 173:1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-2938, 1983;
30 Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., *Proc. Natl. Acad. Sci. U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991;

Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, *Leishmania* spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease.

Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present
5 invention.

Using the proteins of the invention it may also be possible to regulate immune responses in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by
10 suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and
15 persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing
20 high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys
25 the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the
30 molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term

tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

5 The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as
10 described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

15 Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.
20 Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from
25 the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and
30 murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune

response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2

microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated
5 immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated
10 immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without
15 limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al.,
20 J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnoli et al.,
25 Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro*
30 antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek,

D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

5 Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; 10 Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, 15 proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; 20 Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

25

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell 30 lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid

cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., *Experimental Hematology* 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and

Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

5

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and

in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The
5 compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal
10 tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and
15 peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's,
20 Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the
25 invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for
30 generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation

of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., *Endocrinology* 91:562-572, 1972; Ling et al., *Nature* 321:779-782, 1986; Vale et al., *Nature* 321:776-779, 1986; Mason et al., *Nature* 318:659-663, 1985; Forage et al., *Proc. Natl. Acad. Sci. USA* 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. *J. Clin. Invest.* 95:1370-1376, 1995; Lind et al.

APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

5 A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting
10 formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

15 Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

20 A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands,
25 receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant
30 receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static
5 conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

10 Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the
15 inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic
20 inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

25

Cadherin/Tumor Invasion Suppressor Activity

Cadherins are calcium-dependent adhesion molecules that appear to play major roles during development, particularly in defining specific cell types. Loss or alteration of normal cadherin expression can lead to changes in cell adhesion properties linked to
30 tumor growth and metastasis. Cadherin malfunction is also implicated in other human diseases, such as pemphigus vulgaris and pemphigus foliaceus (auto-immune blistering skin diseases), Crohn's disease, and some developmental abnormalities.

The cadherin superfamily includes well over forty members, each with a distinct pattern of expression. All members of the superfamily have in common conserved

extracellular repeats (cadherin domains), but structural differences are found in other parts of the molecule. The cadherin domains bind calcium to form their tertiary structure and thus calcium is required to mediate their adhesion. Only a few amino acids in the first cadherin domain provide the basis for homophilic adhesion; modification of this
5 recognition site can change the specificity of a cadherin so that instead of recognizing only itself, the mutant molecule can now also bind to a different cadherin. In addition, some cadherins engage in heterophilic adhesion with other cadherins.

E-cadherin, one member of the cadherin superfamily, is expressed in epithelial cell types. Pathologically, if E-cadherin expression is lost in a tumor, the malignant cells
10 become invasive and the cancer metastasizes. Transfection of cancer cell lines with polynucleotides expressing E-cadherin has reversed cancer-associated changes by returning altered cell shapes to normal, restoring cells' adhesiveness to each other and to their substrate, decreasing the cell growth rate, and drastically reducing anchorage-independent cell growth. Thus, reintroducing E-cadherin expression reverts carcinomas
15 to a less advanced stage. It is likely that other cadherins have the same invasion suppressor role in carcinomas derived from other tissue types. Therefore, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to treat cancer. Introducing such proteins or polynucleotides into cancer cells can reduce or eliminate the cancerous changes observed
20 in these cells by providing normal cadherin expression.

Cancer cells have also been shown to express cadherins of a different tissue type than their origin, thus allowing these cells to invade and metastasize in a different tissue in the body. Proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be substituted in these cells for the
25 inappropriately expressed cadherins, restoring normal cell adhesive properties and reducing or eliminating the tendency of the cells to metastasize.

Additionally, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to generate antibodies recognizing and binding to cadherins. Such antibodies can be used to block
30 the adhesion of inappropriately expressed tumor-cell cadherins, preventing the cells from forming a tumor elsewhere. Such an anti-cadherin antibody can also be used as a marker for the grade, pathological type, and prognosis of a cancer, i.e. the more progressed the cancer, the less cadherin expression there will be, and this decrease in cadherin expression can be detected by the use of a cadherin-binding antibody.

Fragments of proteins of the present invention with cadherin activity, preferably a polypeptide comprising a decapeptide of the cadherin recognition site, and polynucleotides of the present invention encoding such protein fragments, can also be used to block cadherin function by binding to cadherins and preventing them from binding in ways that produce undesirable effects. Additionally, fragments of proteins of the present invention with cadherin activity, preferably truncated soluble cadherin fragments which have been found to be stable in the circulation of cancer patients, and polynucleotides encoding such protein fragments, can be used to disturb proper cell-cell adhesion.

Assays for cadherin adhesive and invasive suppressor activity include, without limitation, those described in: Hortsch et al. J Biol Chem 270 (32): 18809-18817, 1995; Miyaki et al. Oncogene 11: 2547-2552, 1995; Ozawa et al. Cell 63: 1033-1038, 1990.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via antibody-dependent cell-mediated cytotoxicity (ADCC)). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s);

effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic
5 lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another
10 material or entity which is cross-reactive with such protein.

ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a
15 pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the
20 carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other
25 agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor,
30 thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical

compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in
5 combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If
10 administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical
15 composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is
20 administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention.
25 When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid
30 form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present

invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 µg to about 100 mg (preferably about 0.1mg to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in

R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other

ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and
5 biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions
10 from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of
15 carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to
20 provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in
25 question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to
30 humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of

a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth
5 factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such
10 polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present
15 invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

What is claimed is:

1. An isolated polynucleotide selected from the group consisting of:
 - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;
 - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 29 to nucleotide 253;
 - (c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone ya15_1 deposited under accession number ATCC 98650;
 - (d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;
 - (e) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
 - (f) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2, the fragment comprising eight contiguous amino acids of SEQ ID NO:2;
 - (g) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 65 degrees C, or 4X SSC at 42 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(f); and
 - (h) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 50 degrees C, or 6X SSC at 40 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(f), and that has a length that is at least 25% of the length of SEQ ID NO:1.
2. The polynucleotide of claim 1 wherein said polynucleotide is operably linked to at least one expression control sequence.
3. A host cell transformed with the polynucleotide of claim 2.
4. The host cell of claim 3, wherein said cell is a mammalian cell.
5. A process for producing a protein encoded by the polynucleotide of claim 2, which process comprises:

- (a) growing a culture of the host cell of claim 3 in a suitable culture medium; and
 - (b) purifying said protein from the culture.
- 6. A protein produced according to the process of claim 5.
- 7. An isolated polynucleotide encoding the protein of claim 6.
- 8. The polynucleotide of claim 7, wherein the polynucleotide comprises the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650.
- 9. A protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:2;
 - (b) a fragment of the amino acid sequence of SEQ ID NO:2, the fragment comprising eight contiguous amino acids of SEQ ID NO:2; and
 - (c) the amino acid sequence encoded by the cDNA insert of clone ya15_1 deposited under accession number ATCC 98650;the protein being substantially free from other mammalian proteins.
- 10. The protein of claim 9, wherein said protein comprises the amino acid sequence of SEQ ID NO:2.
- 11. A composition comprising the protein of claim 9 and a pharmaceutically acceptable carrier.
- 12. An isolated polynucleotide selected from the group consisting of:
 - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;
 - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 151 to nucleotide 288;
 - (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 196 to nucleotide 288;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone ya24_1 deposited under accession number ATCC 98650;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone ya24_1 deposited under accession number ATCC 98650;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4, the fragment comprising eight contiguous amino acids of SEQ ID NO:4;

(j) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 65 degrees C, or 4X SSC at 42 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(i); and

(k) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 50 degrees C, or 6X SSC at 40 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(i) and that has a length that is at least 25% of the length of SEQ ID NO:3.

13. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) a fragment of the amino acid sequence of SEQ ID NO:4, the fragment comprising eight contiguous amino acids of SEQ ID NO:4; and

(c) the amino acid sequence encoded by the cDNA insert of clone ya24_1 deposited under accession number ATCC 98650;

the protein being substantially free from other mammalian proteins.

14. An isolated polynucleotide selected from the group consisting of:
- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;
 - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 615 to nucleotide 908;
 - (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 774 to nucleotide 908;
 - (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yb42_1 deposited under accession number ATCC 98650;
 - (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
 - (f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone yb42_1 deposited under accession number ATCC 98650;
 - (g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
 - (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
 - (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6, the fragment comprising eight contiguous amino acids of SEQ ID NO:6;
 - (j) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 65 degrees C, or 4X SSC at 42 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(i); and
 - (k) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 50 degrees C, or 6X SSC at 40 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(i) and that has a length that is at least 25% of the length of SEQ ID NO:5.
15. A protein comprising an amino acid sequence selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:6;

- (b) a fragment of the amino acid sequence of SEQ ID NO:6, the fragment comprising eight contiguous amino acids of SEQ ID NO:6; and
 - (c) the amino acid sequence encoded by the cDNA insert of clone yb42_1 deposited under accession number ATCC 98650;
- the protein being substantially free from other mammalian proteins.

16. An isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 1203 to nucleotide 2327;
- (c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yc9_1 deposited under accession number ATCC 98650;
- (d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650;
- (e) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;
- (f) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8, the fragment comprising eight contiguous amino acids of SEQ ID NO:8;
- (g) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 65 degrees C, or 4X SSC at 42 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(f); and
- (h) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 50 degrees C, or 6X SSC at 40 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(f) and that has a length that is at least 25% of the length of SEQ ID NO:7.

17. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:8;
- (b) a fragment of the amino acid sequence of SEQ ID NO:8, the fragment comprising eight contiguous amino acids of SEQ ID NO:8; and

(c) the amino acid sequence encoded by the cDNA insert of clone yc9_1 deposited under accession number ATCC 98650;
the protein being substantially free from other mammalian proteins.

18. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 230 to nucleotide 823;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 584 to nucleotide 823;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yc19_1 deposited under accession number ATCC 98650;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone yc19_1 deposited under accession number ATCC 98650;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10, the fragment comprising eight contiguous amino acids of SEQ ID NO:10;

(j) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 65 degrees C, or 4X SSC at 42 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(i); and

(k) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 50 degrees C, or 6X SSC at 40 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(i) and that has a length that is at least 25% of the length of SEQ ID NO:9.

19. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10;
- (b) a fragment of the amino acid sequence of SEQ ID NO:10, the fragment comprising eight contiguous amino acids of SEQ ID NO:10; and
- (c) the amino acid sequence encoded by the cDNA insert of clone yc19_1 deposited under accession number ATCC 98650;

the protein being substantially free from other mammalian proteins.

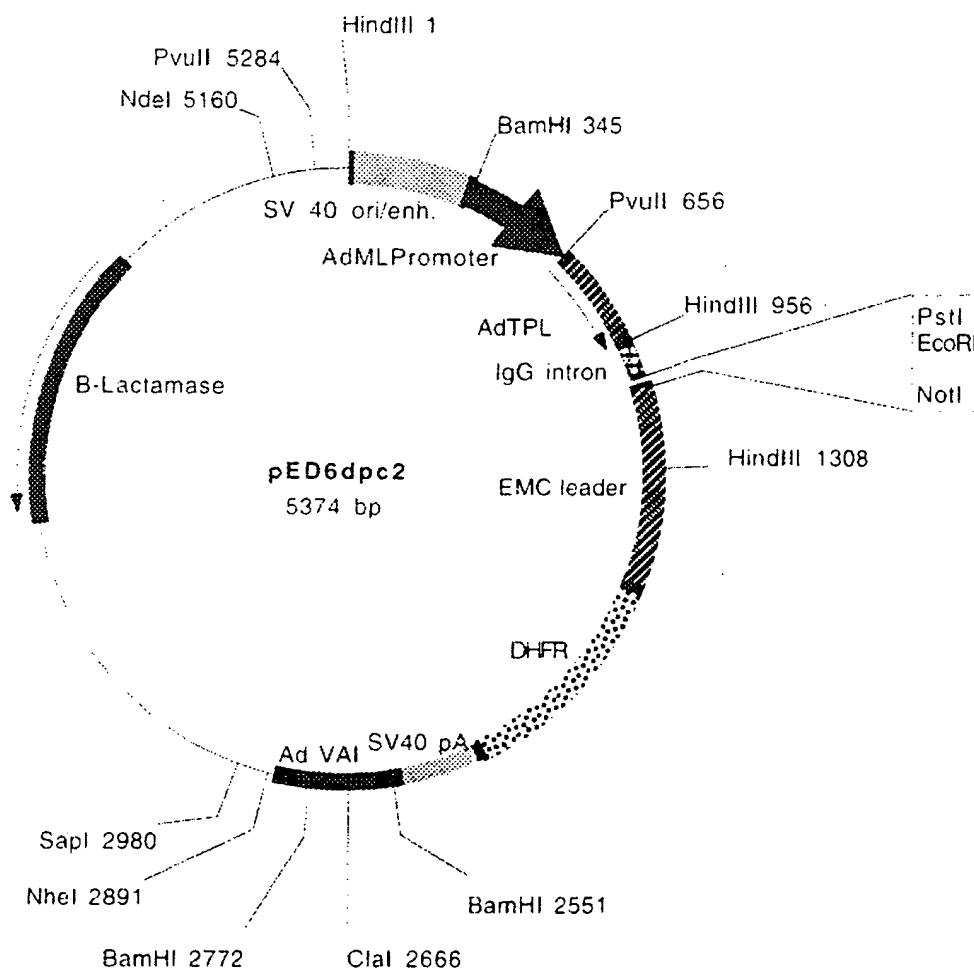
20. An isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 292 to nucleotide 534;
- (c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone yc20_1 deposited under accession number ATCC 98650;
- (d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650;
- (e) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
- (f) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12, the fragment comprising eight contiguous amino acids of SEQ ID NO:12;
- (g) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 65 degrees C, or 4X SSC at 42 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(f); and
- (h) a polynucleotide that hybridizes under conditions at least as stringent as 4X SSC at 50 degrees C, or 6X SSC at 40 degrees C with 50% formamide, to any one of the polynucleotides specified in (a)-(f) and that has a length that is at least 25% of the length of SEQ ID NO:11.

21. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:12;
 - (b) a fragment of the amino acid sequence of SEQ ID NO:12, the fragment comprising eight contiguous amino acids of SEQ ID NO:12; and
 - (c) the amino acid sequence encoded by the cDNA insert of clone yc20_1 deposited under accession number ATCC 98650;
- the protein being substantially free from other mammalian proteins.

FIGURE 1A

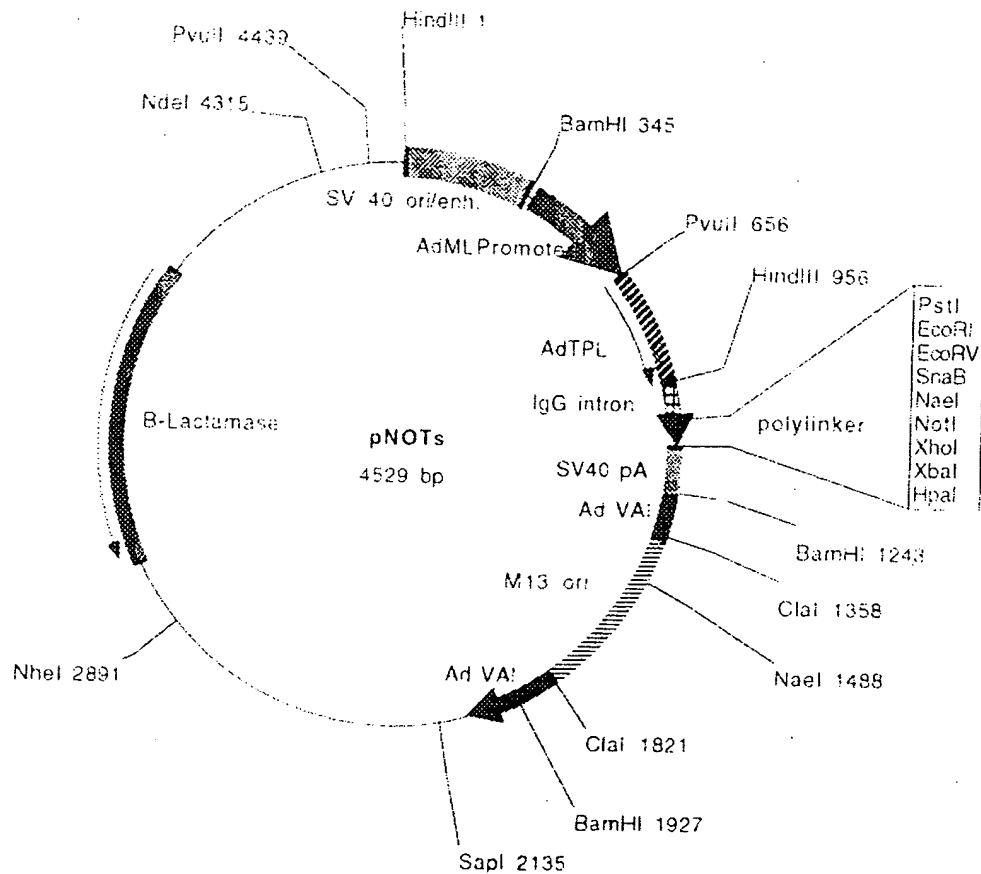


Plasmid name: pED6dpc2

Plasmid size: 5374 bp

Comments/References: pED6dpc2 is derived from pED6dpc1 by insertion of a new polylinker to facilitate cDNA cloning. SST cDNAs are cloned between EcoRI and NotI. pED vectors are described in Kaufman et al.(1991), NAR 19: 4485-4490.

FIGURE 1B



Plasmid name: pNOTs

Plasmid size: 4529 bp

Comments/References: pNOTs is a derivative of pMT2 (Kaufman et al. 1989, Mol. Cell. Biol. 9:1741-1750). DHFR was deleted and a new polylinker was inserted between EcoRI and HpaI. M13 origin of replication was inserted in the Clai site. SST cDNAs are cloned between EcoRI and NotI.

SEQUENCE LISTING

<110> Wong, Gordon G.

Clark, Hilary

Fechtel, Kim

Agostino, Michael J.

Genetics Institute, Inc.

<120> SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM

<130> GI 6300A

<140>

<141>

<160> 19

<170> PatentIn Ver. 2.0

<210> 1

<211> 1051

<212> DNA

<213> Homo sapiens

<400> 1

taggccatga aggccgcttg ctattattat gcaaataagt gttgtattac acatactaata 60
 ttatgcatgg ttagattatg cagatgcac acaaacatgg ttattgaata ataggatggg 120
 gctcattctc tctaccatct ttataagcag ttttaagcaa attctcctgg taccaatgtg 180
 gacattttaa gaccctttac tcaatgaaat gtctctttcg tattcttttg ctttcatagt 240
 taaagccatc agataagtgg aagagaaatc gtccaagttg ttaggttcaa gagtgtagt 300
 ctcacttttc aaattcgtag acttttttgg ttaaatgtaa tcttttcctt atagagaaaa 360
 tctaaaatgc agttgcttgg catgaatgct ggcatttagt gagattttag tgtatatagc 420
 cttgctgctt agctctaggt aacccatcaa attaaaatta cattttcagg atttatagct 480
 cattagaata tttatcttgg taagcttctt attctgtcag taatttctaa acaattcagc 540
 ttggccaatt tgtgaaatcc cctaaaattt tgaaagtga ctcacaagcc ctatgcagta 600
 tatttctcaa acaaatctta gtagaaaact tataagccat ccagtaaaaa ttccaaaggt 660
 tgagaatgta gcaatattct tgagattcct aatgtctaga gtagttaac agtgagattt 720
 gatgggtgat gagtctaaga aatggatttt gccatggcca ggtgcagtg cttacgcctg 780
 taatcccagc actttgggag gccgaggtgg gcgcatcac aggtcaggag attgagacca 840
 tctgtgctaa catggtgaaa cccygtctct actaaaaata caaaaaaaaa aaattagccg 900
 ggcgtggtgg cgggcacctg tagtccctgc taggcagga gaatggcttg aacccgggag 960
 gtggagcttg cagtgaagcc agatcacgcc actgcactcc agcctgggca acagagcaag 1020
 attccgtctc aaaaacaaaa aaaaaaaaaa a 1051

<210> 2

<211> 75

<212> PRT

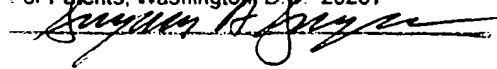
<213> Homo sapiens

<400> 2

Met Gln Ile Ser Val Val Leu His Ile Leu Ile Tyr Ala Trp Leu Asp
 1 5 10 15
 Tyr Ala Asp Ala Ser Gln Thr Trp Leu Leu Asn Asn Arg Met Gly Leu
 20 25 30
 Ile Leu Ser Thr Ile Phe Ile Ser Ser Phe Lys Gln Ile Leu Leu Val
 35 40 45
 Pro Met Trp Thr Phe Lys Asp Pro Leu Leu Asn Glu Met Ser Leu Ser

"Express Mail" mailing label number: Em54025070105Date of Deposit: 11 February 1999

I hereby certify that this paper or fee is being
 deposited with the United States Postal Service
 "Express Mail Post Office to Addressee" service
 under 37 CFR 1.10 on the date indicated above
 and is addressed to the Assistant Commissioner
 For Patents, Washington, D.C. 20231



50

55

60

Tyr Ser Phe Ala Phe Ile Val Lys Ala Ile Arg
 65 70 75

<210> 3
 <211> 711
 <212> DNA
 <213> Homo sapiens

<400> 3
 ttcagggttta gtgctattgt cattgaacac tggatatttc tgtatcatat aaaacattaa 60
 aattcaaata attataagca tttggcaaaa acaagagaaa agaaacttgc catattttac 120
 aagctgcaat tttagaaaag ctttaactta atgatatgtt tatcattgtt ttcttgtecc 180
 aaacttatcc agggccatag aagtatgaat ctaattaaaa cagaaatggg aattattgca 240
 cagaaatggg aaataactaa ttttaaata gtcmaattgg cttcttatta aatacaataa 300
 ttcttatgaa aatcatagta ccctattttc agacacagct gccagtttac acatttctca 360
 gtatcctgaa aggaaaaaag tatagcccca cttatactat gtaaaattac caataaaata 420
 tttttatgac tacagatttt gcatttttgt ttacaactat ttaaagagtt ttatgttgta 480
 tttagaattt caacctagaa accacacagt acttaaattc tcttgggggc tcttgctttc 540
 tcttaaccat ttgtctaata tatatctacc taaaggagac ttctgaattg taaatgaact 600
 taaaaataga atgtggatgc aaaaatatcac ataagacatc atgataacat ttgaagaaaa 660
 aataaaactg tagaccctaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a 711

<210> 4
 <211> 46
 <212> PRT
 <213> Homo sapiens

<220>
 <221> UNSURE
 <222> (42)

<400> 4
 Met Ile Val Leu Ser Leu Phe Ser Cys Pro Lys Leu Ile Gln Gly His
 1 5 10 15
 Arg Ser Met Asn Leu Ile Lys Thr Glu Met Gly Ile Ile Ala Gln Lys
 20 25 30
 Trp Glu Ile Thr Asn Phe Lys Ser Val Xaa Leu Ala Ser Tyr
 35 40 45

<210> 5
 <211> 4529
 <212> DNA
 <213> Homo sapiens

<400> 5
 cacctgggac ctctcacagg tggcatgccc ttccaagacc aggagagaac accacaactt 60
 tgcaggacca gcatctctca gectgectcc tccctttctc catatcaata tgatatggat 120
 gagtactcat ccagcattgg atgatatgga tgaatgggtc tccatcccag agcactgtcc 180
 ctactccagt tccagggcac tcttagtctg ttcttggcac atacagcttt gcatctgtca 240
 gtgtccatgc aaaagccaac acaagctgtg atggactgtg cattgggtag ccaccagagg 300
 taactgccac ttatctatca gtcagccttt gacaggcctc acgtagatac gtacctccca 360
 tcacctccaa gctggaatac actttactca tctgtaaaat gggtagaac atgcctcctt 420
 tgcagarcct ttttgaagat tctgcattta ctagttaaac aactgttcag tacctattat 480
 gggcctagct accggcacat agtaaact cagttacaat agctttaaga aaatctgtct 540

```

tagacaaagg agtttcaaaa ctatgtgaag agtatacaag aagtagtagt ccaaactgtt 600
ttttcagttg tttaatgata tctatgataa agatgttcag ggagaggaa atcttgctg 660
ataactggag taaaacccac tttagatttct atgtccccaa acctctggtg tggtcagggt 720
tggcacctgc cctctcaacc atttgtctca ctgtacatgc atcttgctta tgtcttccac 780
tagagaccat gagggcagag atcagacctt tgtacgttgc acattgctgt cccaggtacc 840
tagcacagca ccttgcccca gtacgcactt catatatatt tcctggacta cggcagggtg 900
gccctttgtg aggggtccac ctgcccactg gcctggcctt taatgtcagg ggagcatttc 960
tgatatatta atgcccctct aattgtgctg cattcgttta cataggcagt gcagcagcct 1020
ggctttggtg tttaaagacc ggattgcagt caaagctctg ctgtttgctg gctatgaact 1080
ccaggtaagt tgccagggtt ctctgagcac taatgataat attacctgcc tcacagggtt 1140
gttttgagag cagcttctgc tgcgtcccca tgcccagaa ttatggaaaa ccattttgtg 1200
aacttcaagt cacttcctct ccatagtaca gatcaatggg agaccagtga tcagggagga 1260
tacaaatcat aaccccaaaa aaggcttctt tgttgtattt tacctcaagt gttttcttag 1320
agaacctcaa caaacacac tattttctag taatcagcca cttatccac catttgctg 1380
aaggaaaact gaggcacgcc ttgatggctt attcagcatc agcagcctca caaaagacag 1440
aagtctcctt tggattagct ttatcatctt tcccttaagc agagatgatg gtgcctttct 1500
cgctgctctg gctcagttaa gaagtcaagt ctgatttatt ggcatccta actatcgttt 1560
cctgaaggcc cgaagccctg taatgctgtt gcttggttcc ccagatttat gaccatggtt 1620
tctggccccc aacaatatct ggagtctggt ttacatatct acttccacca tccaatccaa 1680
atcctcccag gcagcaatca agctggggct gattcctcat ccatttgtgg gctcactttt 1740
tctggcaagt cagccttcat tctccaggac gcttagaagc cgcatgttgg cggtaggattg 1800
tgctacggga aagagtgaat gtgtagcctg cacatttgaa gccttgctgg ggtagagagc 1860
aacgctactg ctctggaat gtccaggcca ctgtagaaa ggccatctta ttccttagaa 1920
gagtctagcc ctgctgcagg cttcagaaac gtgtgctcta agctcgttcc tgcctatttg 1980
aagcagctct ctgaaaagg ctgcagaaac ctgcctttac cctctccctc atcctgctg 2040
gcctttgtca catccttctt ccaagggttc tctgggtggt gtagtaggga ggctgccacc 2100
tggcttttga tgtgggaaag cccagcctct tccctccacc ctcacagcag gacagccaa 2160
cttggatttt ttcatgagga agcaactctt atgagctctc tggttcagct ctctgggtta 2220
aagaccatac tgtgctgttt ctggggcagc acagacctgg ttgatcgtgt ccacaggaa 2280
aacagccttt gggattcatt agtctgggta agaccagccc tgcagcctcc tctgccagct 2340
atagaactat gtgttagagt tgggtctgaa gccagtcgca aaccatccc agatgcgtct 2400
ggctctgttg atgttcaaat gccacctctt cctccaggaa gacttcacca cctcccaa 2460
gctgccttat gggctcctt tctgttccct ctgactctc cccctctatc actatggcca 2520
ttttatagaa ttttaattat tgtctcgggt gtctccacta gactgtaagt atcaagtgtg 2580
tagagacttt tgtttcaact ctgtatcccc aaaattcagc atagttcata gcacatacag 2640
tactctcaat gcctgttgaa tgaataaaga gtctgcttgc atgtaaaact acagtgggtt 2700
ggctctggag acactgttt tgggaattctt ctactgatat ggacagcatc aagatgatgg 2760
gaaatgtatt ggtctgggat ttgttttgcc tacaaagttt cttatgttgg ctcatctcca 2820
actagataaa accctgagtc taggcttccc cttctaaca agctgctata ctttatgga 2880
cctccatttt cctgtctgta aaatgagagt gtgggctagc tagaaaaatt tctgggtgtc 2940
cccttgctgc tttgtaattc tggagtacgt tcggagaaca cccaatgtg acaaaggatc 3000
tggaaattct tcataaaata ttcaggaggg gagacaactc agggaaacatg acaactgtct 3060
ggaattattt gaggggttgt catgtagaag atgaaaagga ttagattatc ttgaaaggcc 3120
ccacagggtt taaataagac caattattaa ttcattcaac aaatatttga gtacctgcca 3180
tgtgcaaggc actattctaa gcaataatga tgcagtaatg aatacaacag acaaaaatct 3240
tgttgccctt atggagttga cattctagtg aacagaaaca attcaaaaac aaacacaatg 3300
gagaggagct tcttgaccc tcaaaacgag ctgcctgtga actattgagc tctttgtgtt 3360
caggaatacg ttagtactt agaaatctgc ttccagtggc tgccttagact aaatgacctt 3420
gttaggtttt ttagcccgag caatcccatg aatctaaggc tagcgaagat gtgtgatctc 3480
tctattctaa caccactaag ccaaatcatg acctcctctc aatggtaact gtcactctaa 3540
aaagtagagt aaggtagatg cagtgggctg tcttatgaa aaggcaccta aaaaatagta 3600
ctttagaagc atgcattgtg gtgggggtct ggggtggagg gttgcttttt cttgagatcc 3660
ctgtgatatt gaggatgaag aaagagaaac acagggggtg ggcaacttcc ttgaaggacc 3720
agagagagtg agtcatgagg aagagaagga ggagacagag aacaggagag ttggggatga 3780
agcctttgat gaggggagtg aggggggca gaagtaggag acagacatga ggaaaccacc 3840
aactgaagtc ccagaaaccc agcctccgtc cctcttaggt cctactgccg tcactctctt 3900
tctgtaaagg tgagagtcta ggtgcagtgt ccagggttcc aaacacaagt tcatactcat 3960
tgactagtgg gatgccact gtgagagcag gaaggagggc tagagagatc taagagggca 4020
gaagccctgc cctgccagta ttttttgctt ccagccacct gctctgttct gagacatgcc 4080
tgagaccagc tgccacagc gggaccaggg tctgtttctg ctgtaaacct tggttccctc 4140

```

```

ctgcattcct gcatgtgggg atgtagacag gaagcctgca ggtatggagc aattgaagca 4200
gagtctagat agagcccaga ctcttactt cccatagtag tattctgcat tttgtagtgc 4260
tgcttatgct tttcagtgtt tccacatcta ttactaata acatcatagt acaacagcaa 4320
gaacatggcc tcagaactca aagttctgga attctaatat tttatttgat tgtcagtatt 4380
tctgtgagat agataaaata ggaattattt cctctgatct gcagggtgagg aaactcagtc 4440
acagggagca catttatcca tacttgtgga gctatagtag aactagtatt aggacaagga 4500
tagccaacat agttaaaaaa aaaaaaaaaa 4529

```

<210> 6

<211> 98

<212> PRT

<213> Homo sapiens

<400> 6

```

Met Ile Ser Met Ile Lys Met Phe Arg Glu Arg Asn Ile Leu Arg Asp
  1             5             10            15

```

```

Asn Trp Ser Lys Thr His Leu Asp Phe Tyr Val Pro Lys Pro Leu Val
      20             25            30

```

```

Trp Ser Gly Leu Ala Pro Ala Leu Ser Thr Ile Cys Leu Thr Val His
      35             40            45

```

```

Ala Ser Cys Leu Cys Leu Pro Leu Glu Thr Met Arg Ala Glu Ile Arg
      50             55            60

```

```

Pro Met Tyr Val Ala His Cys Cys Pro Gln Tyr Leu Ala Gln His Leu
      65             70            75            80

```

```

Ala Pro Val Arg Thr Ser Tyr Ile Phe Pro Gly Leu Arg Gln Val Ser
      85             90            95

```

Pro Leu

<210> 7

<211> 3537

<212> DNA

<213> Homo sapiens

<400> 7

```

aacttagatt tcttaaaact gtatacagat aactaatatt tattttgaaa tagcttttat 60
gttcttggtg ttatgtaata gtcacattaa ttttgctctt ttggctttgt gttgtgttta 120
tttgatgact gttttattcg gtttaatttt ttcttgattt tgcaaattca tttttatcat 180
ctttaagtgg tggagtggcc atttcagctt gaaatatttc actgtcttta taatttcatt 240
ccatgcatgt gttttgtgtt tgtgacggtg tgggtgtatt tagtctatag aagaacatgt 300
ggagcaggtc aacccaaact gcatagcaga agattgccac atcaaggtag tattccaaga 360
tcttgatttt agctcattct ttaaagttca aggagctaac gcctcatctc tgatactaac 420
catgacctgc ccaactggct aactactcag agaactcata gtactcccat accacttcag 480
tggcacaggg gaggagatgc ttcactgtat ctggcttgct ctgtcttgcc cttggttctt 540
ctctttcttg ctccctgtcc catcttcact tgtcactttt tctgttttaa gcatggctgt 600
ttcatcttct tgattctgcc ttttctttcc ttcccatcac caaccctaag ccattgcac 660
ttctctcat cagtctcttt tattcctttc gaagtatccc tctttccaac ttttcttat 720
ttctgtctca ccaccttac ttcaaagggt ggtttctttg cggttttcta tggcttttta 780
ctccactctg tgcctcttaa tgtatacaca ttcttattgc agaataattta tgcacttttg 840
gaagagctaa gagagacata ctactgtata aattactaat aaaaatatat tagccatgtt 900
ttataattaa tcagtatctt accatcacag tggaaattgg ggtgattaca ggacatcaga 960
gaatttaagc aaattttcca aagctagttt tcaacacatt aaagttaact ttaccctttt 1020
ttcttgctgc tgctgctgct gctacatttt cttcttatcc tacatttaaa tcttatggga 1080

```

tgtgcataac tctaaataga gctgtgctgc agcaactaaa actgctgatg aatgttttct 1140
 aggttgaagc tgatctgggc tatccagggtg gaaaggcgaa agtcatccat aaggaatctg 1200
 atatgatcat ggcattttct gtaataaagg caaattgtaa tgaaattgtt ttggcttcaa 1260
 cacatgatgt tcaagaactt gatgttactt ctctactggc ctgtcagtcac tacatatgga 1320
 tcggagaaga atatgacaga gaatccaaaa gtccagatga tgttgattat cgtggttcca 1380
 ctacaactct ttatcaaccc agtgcacat cctattcagc aagtcagggtg catccacctt 1440
 catctctgcc atggctgggc actggacaga ctagcactgg agctagtgtg cttatgaaaa 1500
 ggaatctaca taatgttaag agaattgactt cacacccagt ccatcaatac tatcttacag 1560
 gtgctcagga cggcagtgta cgaatgtttg aatggacgcg gcctcagcaa cttgtctgct 1620
 ttctgcaagc tggcaatgca agagttacta gattatattt taattcacia ggcaacaagt 1680
 gtgggtgttc ggatggagag ggttttctga gtatctggca agttaaccaa actgcatcaa 1740
 atcctaaacc ttatatgagt tggcagtgcc acagtaaagc cacaagtgcac tttgcattta 1800
 ttacctcttc aagtctagtt gccacatctg gacactccaa tgacaataga aatgtttgcc 1860
 tctgggacac attaatatca cccggaaaca gcctcattca tggtttcacg tgccacgac 1920
 atggtgccac ggtactgcag tatgcacca aacagcaact cctaattctg gggggtagga 1980
 aaggacacgt ctgcattttt gacatcaggc aaaggcagct cattcacacg ttccaggccc 2040
 atgactcagc taataaggct ctggccttgg atccctatga ggaatatttt accacaggtt 2100
 cagcagaagg taacataaag gtttgagat tgacaggcca tggcctaatt cattcattta 2160
 aaagtgaaca tgctaagcag tccatatttc gaaacatttg ggctggagtc atgcagattg 2220
 acatcatcca gggcaatcgg ctcttctcct gtggtgcaga tggcacgctg aaaaccaggg 2280
 ttttgcccaa tgcttttaac atccctaaca gaattcttga cattctataa agattggggg 2340
 tttattttta tatacatttc agttaaaagg cacactacag tcatcactag gcaattctgc 2400
 tttctaagca gttgtattga aaacagagaa tctctgtgta gaatttgaat atgaccaag 2460
 ctgagtatta tctaaacagg ttggtggaat gaatgcgcat gtaccttatt atgctgacat 2520
 actaaaaaaa ataaaacctt gtattgtatg aaggatagct attctttaca gcatttagca 2580
 aacctgattc agaaaacatt tgagattagc aaattagtaa cttgaaataa tgaaaaggac 2640
 gtttatacca aattaaggaa gaaaatgttg ctgatttggg tttttcttcc tgttcttacc 2700
 actgactgaa gcctgcctgc agtctcctcc tctgttgaat gaaggataat cataagggtg 2760
 ttgttaggag cgctagacca cctggaaaac tttcttagct gtggagcagt gcgcagtgac 2820
 cagttctctg ctgtgagagg ccgtttccat tctttcctgc tgaatatttt tctgttagt 2880
 gtttatactg agctagtact gtaacttgca aatgagtgc aattttaatg caatgtttta 2940
 ctacacattt gcacattcac attttttgga ctgctagttt ttctatttaa atatttgct 3000
 tcatgttagg aatgtactat gtgaacatga catatttgta gtttaaccaa cacaccttct 3060
 tagtccagtt tagtactttt tcttttctgt tattcaaggt taaacacca aacatttaag 3120
 gatatgttga aactacacca atagagcatt tcatatcata attaaaatga atgttaggct 3180
 tcttgtggcc agttaatagt tgatgagatt ggtgacatta tttattgcca cagcctattg 3240
 tataaactat gcagagttta atatttgctt gtaaaatatt agccaatgtt gtcattattt 3300
 tgatgtattt ccttggttat gacaaaaat atgttgagat actgaaacta atgtctgtgt 3360
 gtttaaatgt ttaccagcaa attgtcttat catgttaatg agaattgttca atgcctgtgt 3420
 ggtaaatagt aaatacaatg gcataaaagt aactttctct gaagatgtga tgttcaggct 3480
 gtgaaatata tatgtaaaag aaaaataaat gttatttggt agaaaaaaa aaaaaaa 3537

<210> 8

<211> 375

<212> PRT

<213> Homo sapiens

<400> 8

Met Ile Met Ala Phe Ser Val Asn Lys Ala Asn Cys Asn Glu Ile Val
 1 5 10 15

Leu Ala Ser Thr His Asp Val Gln Glu Leu Asp Val Thr Ser Leu Leu
 20 25 30

Ala Cys Gln Ser Tyr Ile Trp Ile Gly Glu Glu Tyr Asp Arg Glu Ser
 35 40 45

Lys Ser Ser Asp Asp Val Asp Tyr Arg Gly Ser Thr Thr Thr Leu Tyr
 50 55 60

Gln Pro Ser Ala Thr Ser Tyr Ser Ala Ser Gln Val His Pro Pro Ser
 65 70 75 80
 Ser Leu Pro Trp Leu Gly Thr Gly Gln Thr Ser Thr Gly Ala Ser Val
 85 90 95
 Leu Met Lys Arg Asn Leu His Asn Val Lys Arg Met Thr Ser His Pro
 100 105 110
 Val His Gln Tyr Tyr Leu Thr Gly Ala Gln Asp Gly Ser Val Arg Met
 115 120 125
 Phe Glu Trp Thr Arg Pro Gln Gln Leu Val Cys Phe Arg Gln Ala Gly
 130 135 140
 Asn Ala Arg Val Thr Arg Leu Tyr Phe Asn Ser Gln Gly Asn Lys Cys
 145 150 155 160
 Gly Val Ala Asp Gly Glu Gly Phe Leu Ser Ile Trp Gln Val Asn Gln
 165 170 175
 Thr Ala Ser Asn Pro Lys Pro Tyr Met Ser Trp Gln Cys His Ser Lys
 180 185 190
 Ala Thr Ser Asp Phe Ala Phe Ile Thr Ser Ser Ser Leu Val Ala Thr
 195 200 205
 Ser Gly His Ser Asn Asp Asn Arg Asn Val Cys Leu Trp Asp Thr Leu
 210 215 220
 Ile Ser Pro Gly Asn Ser Leu Ile His Gly Phe Thr Cys His Asp His
 225 230 235 240
 Gly Ala Thr Val Leu Gln Tyr Ala Pro Lys Gln Gln Leu Leu Ile Ser
 245 250 255
 Gly Gly Arg Lys Gly His Val Cys Ile Phe Asp Ile Arg Gln Arg Gln
 260 265 270
 Leu Ile His Thr Phe Gln Ala His Asp Ser Ala Ile Lys Ala Leu Ala
 275 280 285
 Leu Asp Pro Tyr Glu Glu Tyr Phe Thr Thr Gly Ser Ala Glu Gly Asn
 290 295 300
 Ile Lys Val Trp Arg Leu Thr Gly His Gly Leu Ile His Ser Phe Lys
 305 310 315 320
 Ser Glu His Ala Lys Gln Ser Ile Phe Arg Asn Ile Gly Ala Gly Val
 325 330 335
 Met Gln Ile Asp Ile Ile Gln Gly Asn Arg Leu Phe Ser Cys Gly Ala
 340 345 350
 Asp Gly Thr Leu Lys Thr Arg Val Leu Pro Asn Ala Phe Asn Ile Pro
 355 360 365
 Asn Arg Ile Leu Asp Ile Leu
 370 375

<210> 9
 <211> 1466
 <212> DNA
 <213> Homo sapiens

<400> 9
 ccgcgagga ggacggagcc ctaaccgcaa cccgcgcgcg gccgcgcgga tttgatttgt 60
 atccactgtc accagcactg ctacttagg actttctgga tccggaccca ggcagcgcac 120
 actggactct tgaggaagaa ggagactcta attttgatt ccttggtgga ggaaaataaa 180
 aactctggt cttgccgcca acgatgcaag tgtgactgct ggcgtcttca tgagctccag 240
 aggtcacagc acgctaccaaa ggactctcat ggcccctcgg atgatttccg agggagacat 300
 argaggcatt gctcaaatca cctcctctct attcctgggc agaggcagtg tggcctccaa 360
 tcggcacctc ctccaggctc gtggcatcac ctgcattgtt aatgctacca ttgagatccc 420
 taatttcaac tggccccaat ttgagtatgt taaagtgcct ctggctgaca tgccgcatgc 480
 cccatttga ctgtactttg acaccgtggc tgacaagatc cacagtgtga gcaggaagca 540
 cggggccacc ttggtgact gtgctgcagg ggtgagccgc tcagccacgc tgtgtatcgc 600
 gtacctgatg aaattccaca acgtgtgcct gctggaggcg tacaactggg tgaaagcccg 660
 gcgacctgtc atcaggccca acgtaggctt ctggaggcaa ctgatagact acgagcgcca 720
 gctctttggg aagtcgacag ttaaaatggt acagacacct tatggcatag tccccgacgt 780
 ctatgagaag gaggcccgac acctgatgcc ttactggggg atttagtgcc actgaagcct 840
 gcgtcagcag cccgagcggg gccggcatct gctcccgcgc gtctgctccc tctccactct 900
 cttctcaaat ggctgacttc tggttctccc tcaagtgttt tttactactg gtgttcaaat 960
 ttattttaag agataggagg ggaggggaca taaagggaat gcatacattg ctagtccat 1020
 ttttaaaatt aacatttttg aatagtgttt atggaaatct ttagctttta atcattttta 1080
 ccaatttgaa cagtttaata aactggttct gctctcttct gaatctcakg ccttkggcac 1140
 cttggttagt gcaggaggag ctcatgcaa aaatcacttt ggggcctcat taacccttta 1200
 gagacaagyt ttgccccagg ytgccgacca gacagatgyt tagggaaggt tgataccagc 1260
 ttcatgtctc astggattag cctactctt tcccttcccc tccattattt agtgactctg 1320
 taagtaagtt aaatacaccc ttattattta gctgttaagt aactataatg aaatctgctg 1380
 caaaatctct cttggaatcc atgtgccag gattatatta gcattatttt taataaatct 1440
 atatgcttaa caaaaaaaaa aaaaaa 1466

<210> 10
 <211> 198
 <212> PRT
 <213> Homo sapiens

<220>
 <221> UNSURE
 <222> (25)

<400> 10
 Met Ser Ser Arg Gly His Ser Thr Leu Pro Arg Thr Leu Met Ala Pro
 1 5 10 15
 Arg Met Ile Ser Glu Gly Asp Ile Xaa Gly Ile Ala Gln Ile Thr Ser
 20 25 30
 Ser Leu Phe Leu Gly Arg Gly Ser Val Ala Ser Asn Arg His Leu Leu
 35 40 45
 Gln Ala Arg Gly Ile Thr Cys Ile Val Asn Ala Thr Ile Glu Ile Pro
 50 55 60
 Asn Phe Asn Trp Pro Gln Phe Glu Tyr Val Lys Val Pro Leu Ala Asp
 65 70 75 80
 Met Pro His Ala Pro Ile Gly Leu Tyr Phe Asp Thr Val Ala Asp Lys
 85 90 95

Ile His Ser Val Ser Arg Lys His Gly Ala Thr Leu Val His Cys Ala
 100 105 110

Ala Gly Val Ser Arg Ser Ala Thr Leu Cys Ile Ala Tyr Leu Met Lys
 115 120 125

Phe His Asn Val Cys Leu Leu Glu Ala Tyr Asn Trp Val Lys Ala Arg
 130 135 140

Arg Pro Val Ile Arg Pro Asn Val Gly Phe Trp Arg Gln Leu Ile Asp
 145 150 155 160

Tyr Glu Arg Gln Leu Phe Gly Lys Ser Thr Val Lys Met Val Gln Thr
 165 170 175

Pro Tyr Gly Ile Val Pro Asp Val Tyr Glu Lys Glu Ser Arg His Leu
 180 185 190

Met Pro Tyr Trp Gly Ile
 195

<210> 11
 <211> 1521
 <212> DNA
 <213> Homo sapiens

<400> 11
 aatttcttgg gcatttaccg atgccagaag gctaacctgg ggggaggggg gcgcttgtgc 60
 tgggtgaggca cttggataca tactgatgct gcaagttcag gggatttttc ttactcttag 120
 gtttaaccaa gaacactgag cagggaaaaa ccctgccttt cctaactgca tgtatttttt 180
 cctttttgga aaggtggtag agactcagaa gctttccttg ttttcttcag gcctgctccc 240
 agttttctta acagtttctt ttgttgcttt ctctctccct tgttgctttc catggcagta 300
 atcctcctag agtccaagca gtctgttgta tggagcaggg tgtgtgggtt ttctgggccc 360
 atcattatgg ctgcttcaga gtcagaagaa agccataggg cagtagggga gtcctattg 420
 cctarccctt ctccctttgt ggctccact ctagctgcct atttttgtc atcagctggg 480
 gagtcagat gggccagcag ttctccctcc ctaagccctt gctactttat ggggttagctt 540
 tgcaggtttg gtggcttgag ggtgggggg aactcaccac tgccaggtaa ctccctgaag 600
 ggtgggagtg gattatcttc taggctctta cccgcggtag ggaagggcat caacactgtc 660
 ttccttccat tctccttttc cccatcccat ttagtgctgc cacagggcag aagcacacaa 720
 accaaccaca cagtctctga cttctcctaa gcactttgag ttgttgaatg gggctcaggg 780
 gcaagagttt ttgctgcctt cccagcgtg gtcacagggg tattgaactg cctgcacttg 840
 tttctcatgc aactccagca ttttccccag aagttgaact atggatagca gcttggtatg 900
 gatttcctaa atcttaacat ttgaagcagc ttcttgaggc tggcaactat cctggtttct 960
 gtcttgagg ggttggtttg tttgctgggg cccaacgtct gtcccaagtg gtggggtgag 1020
 agtaagttaa ctttggtgcc aggtgagagg tgggggctct ttgcttagac tccctatcat 1080
 ggaaagattg gagttttcta tgcagggcac tggggaaaag gattgctgat tctgactgac 1140
 cctgatcaga gagattagga ttgtattttg acataggatt tggaaccat ctaaagtgtg 1200
 aagttccctg agacrgctct ccagctgctg agcctgcgcc aggggytaag cagcccctaa 1260
 tgagaggtct tgcctccctt cccacctcgc caatgttggt ttgctgcct ttttgatttg 1320
 tatctctgt tatagacatt ttttraaaac gatttctct ttcattgtgc acaagtgtg 1380
 agagtctgag gccccatttc tgctgtgtat atatatcctg actcggggct tttattcagc 1440
 aaactgttca ttcttctgtc agacaatgtc atattcaact ctgttcatat taaaccactg 1500
 tgaagcaaaa aaaaaaaaaa a 1521

<210> 12
 <211> 81
 <212> PRT
 <213> Homo sapiens

<220>

<221> UNSURE

<222> (45)

<400> 12

Met Ala Val Ile Leu Leu Glu Ser Lys Gln Ser Val Val Trp Ser Arg
 1 5 10 15

Val Cys Gly Phe Ser Gly Pro Ile Ile Met Ala Ala Ser Glu Ser Glu
 20 25 30

Glu Ser His Arg Ala Val Gly Glu Leu Leu Leu Pro Xaa Pro Ser Pro
 35 40 45

Phe Val Ala Pro Thr Leu Ala Ala Tyr Phe Cys Ser Ser Ala Gly Glu
 50 55 60

Ser Val Trp Ala Ser Ser Ser Pro Ser Leu Ser Pro Cys Tyr Phe Met
 65 70 75 80

Gly

<210> 13

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide

<400> 13

ggatggggct cattctctc

19

<210> 14

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide

<400> 14

gaaatgtgta aactggcagc

20

<210> 15

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide

<400> 15

gaatgcagca caattagagg g

21

<210> 16

<211> 21

<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 16
ctgcttagaa agcagaattg c

21

<210> 17
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 17
tgtgactagc aatgtatgc

19

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 18
tcagaagaaa gccatagggc

20

<210> 19
<211> 113
<212> PRT
<213> Homo sapiens

<400> 19
Met Ser Arg Pro Leu Leu Glu Arg Pro Ser Tyr Ser Leu Glu Glu Ser
1 5 10 15

Ser Pro Ala Ala Gly Phe Arg Asn Val Cys Ser Lys Leu Val Pro Ala
20 25 30

His Leu Lys Gln Ser Ser Glu Lys Val Cys Arg Asn Leu Pro Leu Pro
35 40 45

Ser Pro Ser Ser Cys Val Ala Phe Val Thr Ser Phe Leu Gln Gly Ser
50 55 60

Ser Gly Gly Gly Ser Arg Glu Ala Ala Thr Trp Leu Cys Met Trp Glu
65 70 75 80

Ser Pro Ala Ser Ser Leu His Pro His Ser Arg Thr Ala Lys Leu Gly
85 90 95

Phe Phe His Glu Glu Ala Thr Leu Met Ser Ser Leu Val Gln Leu Ser
100 105 110

Gly

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/02898

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 530/350; 514/2; 435/69.1, 252.3, 254.11, 325, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 514/2; 435/69.1, 252.3, 254.11, 325, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Biosis, Medline, WPI

search terms: secreted, soluble, cytokines, lymphokines, interleukins, interferons.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Database N.A. on (MPsrch), Accession No. AQ037466, ADAMS, M.D. et al. "Use of a Random BAC END Sequence Database for Sequence-Ready Map Building", abstract, 10 July 1998.	1-11
A	US 5,641,657 A (RUBEN et al.) 24 June 1997, columns 14-16.	1-11
A	US 5,654,276 A (BARRETT et al.) 05 August 1997, columns 13-15.	1-11
A,P	US 5,786,323 A (NAKAHATA) 28 July 1998, columns 3-7.	1-11
A,P	US 5,767,064 A (SIMS et al.) 16 June 1998, columns 16, 17.	1-11

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

21 APRIL 1999

Date of mailing of the international search report

13 MAY 1999

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT

Authorized officer

 JOYCE BRIDGERS
 PARALEGAL SPECIALIST

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/02898

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/02898

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6): C07K 14/52, 14/475; C12N 15/12, 15/63, 15/70, 15/79, 15/79; A61K 38/17

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-11, drawn to polynucleotide (SEQ ID NO: 1) and the encoded polypeptide (SEQ ID NO: 2).

Group II, claims 12 and 13, drawn to polynucleotide (SEQ ID NO: 3) and its encoded polypeptide (SEQ ID NO: 4).

Group III, claims 14 and 15, drawn to polynucleotide (SEQ ID NO: 5) and its encoded polypeptide (SEQ ID NO: 6).

Group IV, claims 16 and 17, drawn to polynucleotide (SEQ ID NO: 7) and its encoded polypeptide (SEQ ID NO: 8).

Group V, claims 18 and 19, drawn to polynucleotide (SEQ ID NO: 9) and its encoded polypeptide (SEQ ID NO: 10).

Group VI, claims 20 and 21, drawn to polynucleotide (SEQ ID NO: 11) and its encoded polypeptide (SEQ ID NO: 12).

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of each Group is the polynucleotide sequence and encoding amino acid sequence. The polynucleotide sequence of SEQ ID NO: 1, 3, 5, 7, and 11 are structurally and functionally distinct sequences. The encoded polypeptide having SEQ ID NO: 2, 4, 6, 8, 10, and 12 are structurally and functionally distinct polypeptides. The special technical feature of each group is not the same as or does not correspond to the special technical feature of any other group because neither the polynucleotides nor the polypeptides share the same sequences. The groups are not linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.